REMARKS

1. Status of claims

After entry of the above amendment to the claims, claims 1-6, 17-18, 22-23, and 35 have been withdrawn from consideration and claims 7-10, 12-16, 19-21, and 24-34 are pending and under consideration.

2. Support for amendment

The amendment to the claims finds support in the specification at p. 5, line 13; p. 8, line 19 to p. 9, line 17; p. 12, lines 22-23; p. 14, line 30 to p. 15, line 5; p. 17, lines 5-28; p. 28, line 27 to p. 29, line 4; p. 36, line 20; and p. 47, line 14. No new matter has been added by this amendment.

3. Election/restriction

Applicants recognize the finality of the restriction requirement previously imposed.

4. Objections to the specification

The Examiner objected to the specification for containing the phrase "mineral medium" instead of "minimal medium" at p, 7, line 24; p. 8, lines 4, 11, 21, and 31; and p. 9, lines 10-11. By the above amendment to the specification, the phrase has been corrected and Applicants submit the basis for this objection has been removed.

5. Claim rejections under 35 U.S.C. §112

The Examiner rejected claims 9-10, 18-21, 25-26, 31, and 34 under 35 U.S.C. §112, second paragraph, as being indefinite. Specifically, the Examiner found the identification of various fungal, plant, and animal species in claims 9-10, 19-21, and 26 and the use of various phrases in claims 18, 25, 31, and 34 to be vague.

In light of the above amendment to the claims and the following remarks, Applicants submit the basis for these rejections have been removed. The various species have been identified by full genus and species name except when recited by a claim dependent on a claim in which the full genus and species name are given; *e.g.*, claim 10 depends on claim 9, in which the term "*Saccharomyces cerevisiae*" is recited, and therefore Applicants submit there is no ambiguity in claim 10 for retaining the term "*S. cerevisiae*."

Concerning the phrase "promoter active in yeast" of claims 18 and 25, Applicants point to the specification at p. 15, lines 4-5, where promoters are characterized as being "homologous or heterologous, constitutive, inducible or repressible." From this, the skilled artisan will understand that a "promoter active in yeast" is one which is constitutively, inducibly, or repressibly active in yeast. Therefore, Applicants submit the phrase and claims reciting it are clear.

Concerning the phrase "wherein the recombinant yeast accumulates L-ascorbic acid in the medium at levels greater than background" of claim 31, Applicants point to the specification at p. 18, lines 1-9. From the cited passage, the skilled artisan will recognize that a background level of L-ascorbic acid in the medium refers to the level of extracellular L-ascorbic acid generated by a yeast that is either nonrecombinant or transformed solely with a gene encoding LGDH. The skilled artisan will also recognize the appropriate control for a given yeast transformed with at least one gene encoding an enzyme other than LGDH is a yeast of the same

strain that is either nonrecombinant or transformed solely with a gene encoding LGDH. Therefore, Applicants submit the phrase and the claim reciting it are clear.

Concerning the phrase "stabilizing ascorbic acid" of claim 34, Applicants point to the specification at p. 28, line 27 to p. 29, line 4, p. 4, line 5, and Figure 2. From the cited passages, the skilled artisan will understand ascorbic acid under aerobic conditions will undergo oxidation and the level of ascorbic acid will drop (Figure 2B). From Figure 2A, it is clear that ascorbic acid levels under aerobic conditions in a culture containing yeast will drop very little (such as less than about 15-20% over 2-7 days), and that "stabilizing ascorbic acid" means "creating conditions under which ascorbic acid levels will drop very little." Therefore, Applicants submit the phrase and the claim reciting it are clear.

Also, the Examiner rejected claims 12-14 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. Specifically, the Examiner alleged that the specification did not reasonably convey to the skilled artisan that the inventors had possession of the claimed invention, specifically relating to D-arabinono-1,4-lactone oxidase (ALO) having at least about 70% similarity or identity with SEQ ID NO:5 or 7 or coding regions encoding ALO and having at least about 70% identity with SEQ ID NO:6 or 8. Applicants traverse this rejection.

The methodology for determining compliance with the written description requirement is given at MPEP 2163.II.A *ff.* First, what each claim as a whole covers is to be determined. Second, the entire application is to be reviewed to understand how it provides support for the claimed invention and each element or step thereof. Third, whether there is sufficient written description to inform the skilled artisan that the applicant was in possession of the claimed invention as a whole at the time the application was filed is to be determined. In the present

situation, the claims in question are original claims (identical to those originally presented in the parent application) drawn to a genus.

ALO enzyme is described at p. 5, lines 18-21 and p. 25, lines 1-5. The skilled artisan would also have been aware of references known in the art at the time of filing of the parent application, such as Huh *et al.*, *Eur. J. Biochem.* 225:1073-1079 (1994); Lee *et al.*, *App. Env. Microb.* 65:4685-4687 (1999). From these, it is clear that the functional term "ALO" refers to an oxidoreductase capable of acting on the CH-OH group of D-arabinono-1,4-lactone with oxygen as an acceptor, EC 1.1.3.37. "Similarity" and "identity" are also well known terms to the skilled artisan, especially in view of description of the CLUSTAL program at pp. 13-14. The plain meaning of "at least about 70%" is apparent.

Applicants will now consider the Examiner's performance of the third step of the methodology. One way in which an adequate written description may be shown is by structural chemical formulae. Another way is by any description of sufficient, relevant, identifying characteristics. Applicants have shown an adequate written description in both ways.

The phrases "at least about 70% similarity to SEQ ID NO:XX" or "at least about 70% identity to SEQ ID NO:XX" are exactly equivalent to a list of sequences (with the ordinary skilled artisan understanding an amino acid or nucleic acid sequence as a "structural chemical formula") differing from SEQ ID NO:XX by 0 amino acids/nucleic acids, 1 amino acids/nucleic acids (of which there are *n* such sequences, wherein *n* is the length of the sequence of SEQ ID NO:XX), 2 amino acids/nucleic acids (of which there are $n*(n-1)$ such sequences, wherein *n* is the length of the sequence of SEQ ID NO:XX), … to about 30% * *n* amino acids/nucleic acids (of which there is a vast number of sequences for values of *n* common for the entire amino acid sequence of proteins). The phrases "at least about 70% similarity to SEQ ID NO:XX" or "at

least about 70% identity to SEQ ID NO:XX", being vastly more concise while providing equivalent information, are used solely as a convenience for the ordinary skilled artisan reading the description and claims.

Concerning a description of sufficient, relevant, identifying characteristics, these characteristics are given by *Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 323 F.3d 956, 63 USPQ2d 1609 (Fed. Cir. 2002) as complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination thereof. The present claims recite ALOs, which the skilled artisan would understand refers to enzymes having the functional characteristics of being oxidoreductases capable of acting on the CH-OH group of D-arabinono-1,4-lactone with oxygen as an acceptor. These functional characteristics are coupled to a disclosed correlation between function and structure, namely, the levels of similarity or identity to SEQ ID NO:5 or 7 recited by the claims. The claims do *not* recite that every protein having at least about 70% similarity or at least about 70% identity to SEQ ID NO:5 or 7 or being encoded by a coding region having at least about 70% identity with SEQ ID NO:6 or 8 is an ALO, i.e., has the functional characteristics discussed above. Rather, the claims recite proteins having both the functional characteristics of ALO and a structure having at least about 70% similarity or at least about 70% identity to SEQ ID NO:5 or 7 or being encoded by a coding region having a structure having at least about 70% identity with SEQ ID NO:6 or 8.

Claims drawn to a genus require a written description of a "representative number of species." There is no *per se* rule regarding how many species constitute a representative number. From *In re Herschler*, 591 F.2d 693, 200 USPQ 711 (CCPA 1979), it is clear that a genus "must have a corresponding written description only so specific as to lead one having ordinary skill in

the art to that [genus]." In view of *Herschler*, there is no need for a written description more specific than that required to lead the ordinary skilled artisan to the genus as a whole. The genera of the present claims are ALOs having at least about 70% similarity or at least about 70% identity to SEQ ID NO:5 or 7 or being encoded by coding regions having at least about 70% identity to SEQ ID NO:6 or 8. Four specific species are presented: an ALO having 100% identity to SEQ ID NO:5, an ALO having 100% identity to SEQ ID NO:7, a coding region having 100% identity to SEQ ID NO:6, and a coding region having 100% identity to SEQ ID NO:6. Turning to the protein, the species is not *a protein* having at least about 70% similarity or at least about 70% identity to SEQ ID NO:5 or 7, but rather *an ALO* having at least about 70% similarity or at least about 70% identity to SEQ ID NO:5 or 7. Analogous reasoning holds for the species and genus of coding regions.

For at least these reasons, an adequate written description is provided, and Applicants request this rejection of claims 12-14 be withdrawn.

6. Provisional claim rejections under obviousness-type double patenting

The Examiner provisionally rejected claims 7-14, 18-21, and 24-33 under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 1-15 of U.S. Pat. No. 6,630,330. The Examiner also provisionally rejected claims 7 and 11-14 under the judicially-created doctrine of obviousness-type double patenting as being unpatentable over claims 12-14 of U.S. 10/606,300. Applicants request deferring substantive response to this rejection (such as the filing of a terminal disclaimer) until such time as claims are allowed in either the present case or U.S. Pat. Appl. 10/606,302.

7. Claim rejections under 35 U.S.C. §102

The Examiner rejected claims 7-8, 28, 31-32, and 34 under 35 U.S.C. §102(b) as being anticipated by Roland *et al.*, WO 85/01745 ("Roland"). In view of the above amendment and following remarks, Applicants request this rejection be withdrawn.

Roland discusses mutated yeast that can be produced by recombinant DNA techniques and used to produce L-ascorbic acid (p. 5, lines 11-18; p. 39, lines 5-9). Roland does not identify any specific genes from any specific sources to be used in such recombinant DNA techniques. Therefore, Roland does not recite every element of the method recited by claim 7, as amended, and all claims dependent thereon. Further, Roland does not provide an enabling disclosure of the method recited by claim 7, as amended, and all claims dependent thereon. As for claim 34, the passage cited by the Examiner at p. 14, lines 4-7 of Roland does not indicate that Roland possessed a method of stabilizing ascorbic acid in a medium by culturing a yeast in the medium, and Roland therefore does not anticipate claim 34.

The Examiner also rejected claims 7-8, 28-32, and 34 under 35 U.S.C. §102(e) as being anticipated by Berry *et al.*, US 2002/0012979 ("Berry"). In view of the above amendment and following remarks, Applicants request this rejection be withdrawn.

Berry teaches a method of producing L-ascorbic acid by fermentation of a genetically modified microorganism, such as a *Saccharomyces* yeast, genetically modified to have increased action of, *inter alia*, L-galactose dehydrogenase and l-galatono-γ-lactone dehydrogenase [0013, 0034]. Berry does not teach methods involving genetically modified yeast transformed with coding regions encoding D-arabinose dehydrogenase (ARA), D-arabinono-1,4-lactone oxidase (ALO), or L-gulono-1,4-lactone oxidase (RGLO), as recited by claim 7 and all claims dependent thereon. As for claim 34, Berry teaches that an ascorbic acid-containing medium containing a

microorganism culture can be manipulated to better maintain or enhance ascorbic acid production by lowering the pH or rendering the environment microaerobic [0123-0124]. Berry does not guide the skilled artisan to the use of yeast as opposed to other microorganisms in this context. In contrast, claim 34 recites that ascorbic acid can be stabilized in an aerobic medium. Berry therefore does not anticipate any of claims 7, 34, or claims dependent thereon.

8. Claim rejections under 35 U.S.C. §103

The Examiner rejected claims 7-9, 11-15, 18, 20-21, 24, and 27-30 under 35 U.S.C. §103(a) as being unpatentable over Lee *et al.*, *App. Env. Microb.* 65:4685-4687 (1999) ("Lee") and Huh *et al.*, *Molec. Microbiol.* 30:895-903 (1998) ("Huh"). Applicants traverse this rejection.

Lee teaches the production of D-erythroascorbic acid and L-ascorbic acid in the bacterium *Escherichia coli* transformed with the *S. cerevisiae* coding region encoding D-arabinono-1,4-lactone oxidase (ALO) by culturing the transformed bacterium in a medium containing D-arabinono-1,4-lactone or L-galactono-1,4-lactone (paragraph bridging pp. 4686-87). The D-erythroascorbic acid and L-ascorbic acid produced by the transformed bacterium were quantified by HPLC of cell extracts (Fig. 3 caption). The transformed bacterium produced about 31-fold more D-erythroascorbic acid than wild type *S. cerevisiae* (paragraph bridging pp. 4686-87).

Huh teaches the production of D-erythroascorbic acid in both wild type, *alo1* disruptant, and *ALO1* overexpressant *S. cerevisiae* (p. 897, second column, first full paragraph). The *ALO1* overexpressant produced about 7-fold more D-erythroascorbic acid than wild type (paragraph bridging pp. 897-898). D-erythroascorbic acid produced by the yeast were quantified by HPLC of cell extracts (Fig. 3 caption). Huh "could not detect [L-ascorbic acid] in all the cells" and Fig. 3E shows the *ALO1* overexpressant produced no L-ascorbic acid (paragraph bridging pp. 897-

898 and Fig. 3E). Huh cites earlier work as indicating that "yeast cells can synthesize [L-ascorbic acid] when L-galactono-1,4-lactone is added to the cells extraneously." However, the earlier work cited is Huh *et al.*, *Eur. J. Biochem.* 225:1073-1079 (1994) ("Huh 1994"), which reports that *Candida albicans* yeast, not *S. cerevisiae*, can synthesize a miniscule amount of presumed L-ascorbic acid when incubated in a large excess of L-galactono-1,4-lactone (Huh 1994, p. 1075, second column, second paragraph, and Figure 1, particularly 1A, 1F). Huh 1994, Figure 1F, shows that the *C. albicans* incubated with a large excess of L-galactono-1,4-lactone produced about 3-fold more D-erythroascorbic acid than L-ascorbic acid.

The Examiner alleges that the skilled artisan would have found it obvious to use a recombinant overexpressant yeast as taught by Huh in the method of Lee. This allegation is improperly drawn. First, there is no motivation to use the *ALO1* overexpressant *S. cerevisiae* of Huh in the method of Lee. Lee reports that *E. coli* expressing ALO from *S. cerevisiae* produces about 31-fold more D-erythroascorbic acid than wild type *S. cerevisiae*. Huh's *ALO1* overexpressant produces about 7-fold more D-erythroascorbic acid than wild type *S. cerevisiae*. The skilled artisan considering modifications to the method of Lee would not be motivated to use a recombinant yeast in place of the recombinant bacterium of Lee given that the recombinant yeast produces about 4-fold less D-erythroascorbic acid than does the recombinant bacterium.

Second, the Examiner's allegation regarding Huh's observation that "*S. cerevisiae* has been recognized as a good model system for understanding the biology of eukaryotic organisms" represents use of the clearly erroneous "obvious to try" rationale. Huh's observation would suggest only that whatever the results of overexpressing ALO in *S. cerevisiae*, these results would provide a basis for considering likely results of overexpressing ALO in other eukaryotic

organisms. Huh's observation does not suggest that the results of overexpressing ALO in *S. cerevisiae* would be readily predictable from the results of expressing ALO in *E. coli*.

Third, the Examiner's allegation that Huh's citing of Huh 1994 suggests that incubation of ALO overexpressant yeast in the presence of L-galactono-1,4-lactone would lead to production of L-ascorbic acid is erroneous. Huh 1994 teaches that *C. albicans* ALO has a relatively high substrate specificity for L-galactono-1,4-lactone (p. 1077, Table 2), which manifests in the overproduction of D-erythroascorbic acid relative to L-ascorbic acid (Figure 1F). The skilled artisan would conclude that an ALO overexpressant yeast would produce even more D-erythroascorbic acid and would not be a good candidate for producing L-ascorbic acid.

For at least these reasons, Applicants request this rejection of claims 7-9, 11-15, 18, 20-21, 24, and 27-30 be withdrawn.

9. Conclusion

Applicants submit all pending claims are in condition for allowance. The Examiner is invited to contact the undersigned patent agent at (713) 934-4065 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

WILLIAMS, MORGAN & AMERSON, P.C.
CUSTOMER NO. 23720

March 24, 2006

/Raymund F. Eich/
Raymund F. Eich, Ph.D.
Reg. No. 42,508
10333 Richmond, Suite 1100
Houston, Texas 77042
(713) 934-4065
(713) 934-7011 (fax)

AGENT FOR APPLICANTS